Competent cells

1. **Plate Top10 cells and incubate at 37ºC overnight**
2. **Pick one colony, inoculate in LB media and incubate overnight while shaking at 37ºC**
3. **Dilute the culture in fresh medium, and continue the incubation until the OD_{600}= 0.4-0.6**
4. **Centrifugation 5 min. at 4000 rpm.**
5. **Pellet cells and resuspend in 100mM of $CaCl_2$ solution**
6. **Incubate on ice for 20 min**
7. **Centrifugation 5 min at 3000 rpm**
8. **Pellet cells and suspend again in 100mM $CaCl_2$ solution**
9. **Incubate on ice for 60 min**
10. **Centrifugation 5 min at 3000 rpm**
11. **Add a solution of 100mM $CaCl_2$ + 40% glycerol**
12. **Store immediately at -80ºC**

Electrocompetent cells

1. **Grow single Escherichia coli colony in LB overnight, at 37ºC and 200 rpm**
2. **Dilute the overnight culture 1:100 in fresh LB medium and grow at 37ºC and 200 rpm, until OD600 is between 0.4 and 0.6**
3. **Place the culture on ice for 15 minutes**
4. **Harvest the cells by centrifugation (5 minutes at 4000 rpm at 4ºC) and resuspend in ice cold Mili-Q water, to the volume of the original culture**
5. **Repeat the last washing step with Mili-Q water 2 times**
6. **Resuspend cells in equal volume as cell pellet ice cold 10% glycerol**
7. **Make aliquots of 40 µL and store at -80ºC**

Transformation

8. **Take the competent cells from the storage at -80ºC and leave them on ice for 10-15 min**
9. **Add 1-2 µL of plasmid solution to the 50 µL cell tube**
10. **Incubate on ice for 30 min**
11. **Heat-shock the cells at 42ºC for 45s**
12. **Incubate on ice for 2 min**
13. **Add 500 µL of LB media and incubate 60 min at 37ºC**
14. **Plate the cultures on agar plate**

Electroporation

1. **Use 40 µL electrocompetent cells for electroporation**
2. **Add 1-10 ng of plasmid DNA or 1 µL of ligation product to the cells**
3. **Transfer cells + DNA to 2mm electroporation cuvette**
4. **Electroporate at 2500 V, with a pulse of 5-6 ms**

Cell cultures

1. **Pick a single colony from a plate or cryostock**
2. **Put the colony in a 50 mL sterile tube and add 5-10 mL of LB fresh medium**
3. **Put the tube to incubate for at least 16 h, at 37ºC and 200 rpm**

DNA Electrophoresis

Gel making

1. **Prepare 200 mL of TAE buffer**
2. **Mix the TAE solution with 2g of agarose (for 1%)**
3. **Heat the solution to boiling, and then cool it to 50ºC aprox.**
4. **Add 5 µL of Ethidium bromide to the solution**
5. **Pour the solution in the electrophoresis vessel. Apply the combs.**
6. **Let it polymerize, and then cover it with TAE**

Gel running

7. **Add 1/6 of total volume of Loading buffer to every DNA sample.**
8. **Remove the combs from the gel, and pipette DNA samples and DNA ladder**
9. **Run at 100-130V for 30-60 min (depends on the fragments)**

Ligation

1. **Add 20-100 ng of vector DNA (can be calculated from the DNA concentration in the sample)**
2. **Add X ng of insert DNA. X is calculated using the length of both vector and insert and the molar ratio desired.**
3. **Add 2µL of ligation buffer**
4. **Add MQ water to set the final volume to 15-20**
5. **Add 1 µL of T4 ligase (always at the end to keep the enzyme in optimal conditions)**
6. **Incubate for at least 3 hours at 16ºC**

Cell Cryostock

1. **Take 1.5 mL from a freshly grown culture and put it in a 1.5 mL tube**
2. **Spin the tube for 10 min at 2000 rpm**
3. **Decant the supernatant without disturbing the pellet**
4. **Add 0.5 mL of LB media and 0.5 mL of glycerol 80% solution**
5. **Mix by vortexing**
6. **Save in the -80ºC freezer**

Restriction (NEB enzymes)

1. **Add 1 µg of DNA (can be calculated from concentration in the sample)**
2. **Add 5 µL of NEB buffer**
3. **Add 1 µL of restriction enzyme 1**
4. **Add 1 µL of restriction enzyme 2**
5. **Add MQ water to set the final volume at 50 µL**
6. **Mix the solution by flicking the tube**
7. **Spin-down in a microcentrifuge for 15 s**
8. **Incubate at 37ºC for 1-2 hours**

Plasmid isolation (from Promega PureYield ® Kit)

1. **Add 1.5 mL of bacterial culture in LB medium to a 1.5 mL micro-centrifuge tube. Centrifuge that tube at max speed for 3 min**
2. **Remove the supernatant, and add 600 µL of MQ water to the pellet**
3. **Add 100 µL of Cell Lysis Buffer, and mix by inverting 6 times. The color change to blue indicates complete lysis**
4. **Add 350 µL of cold (4-8ºC) Neutralization Buffer, and mix by inverting the tube. The color change to yellow indicates total neutralization**
5. **Centrifugate at maximum speed for 3 minutes, and transfer the supernatant to a PureYield Minicolumn**
6. **Place the minicolumn into a PureYield Collection Tube and centrifuge at maximum speed for 15 seconds**
7. **Discard the flowthrough and place the minicolumn again into the same PureYield Collection tube**
8. **Add 200 µL of Endotoxin Removal Wash to the minicolumn. Centrifuge at maximum speed for 15 seconds. Do not empty the Collection Tube now**
9. **Add 400 µL of Column Wash Solution to the minicolumn, and centrifuge at maximum speed for 30 seconds**
10. **Transfer the minicolumn to a clean 1.5 mL tube, and 30 µL of hot (50ºC, pre-warmed) MQ water directly to the minicolumn matrix. Let stand for 5 minutes at room temperature**
11. **Centrifuge at maximum speed in a microcentrifuge for 15 seconds to elute plasmidic DNA. Cap the tube, and store the DNA solution at -20 ºC (or use it directly for cloning experiments)**

Gel isolation (from Promega Wizard ® Kit)

1. **Weigh a 1.5 mL microcentrifuge tube for each DNA fragment to be isolated, and record the weight**
2. **Visualize the DNA in the agarose gel using a long-wavelength UV lamp and an intercalating dye (Ethidium bromide). Irradiate the gel the minimum possible time to reduce nicking**
3. **Excise the DNA fragment of interest in a minimal volume of agarose using a clean scalpel or razor blade. Transfer the gel slice to a weighted 1.5 mL tube and record the weight, again. Subtract the previously measured tube weight to obtain the weight of the gel slice containing the DNA fragment**
4. **Add Membrane Binding Solution at a ratio of 10 µL of solution per 10 mg of agarose gel slice**
5. **Vortex the mixture and incubate at 50-65ºC for 10 minutes, or until the gel slice is completely dissolve in the liquid. You can vortex the tube every few minutes to increase the rate of agarose melting**
6. **Centrifuge the tube briefly at room temperature to ensure the contents are at the bottom of the tube. Once the agarose gel is melted, the gel will not re-solidify at room temperature**
7. **Place one SV Minicolumn in a Collection Tube for each dissolved gel slice**
8. **Transfer the dissolved gel mixture to the SV minicolumn assembly and incubate for 1 minute at room temperature**
9. **Centrifuge the SV Minicolumn assembly in a microcentrifuge at max speed for 1 minute. Remove the SV Minicolumn from the Spin Column assembly and discard the liquid in the Collection Tube. Return the SV Minicolumn to the Collection Tube afterwards**
10. **Wash the column by adding 700 µL of Membrane Wash Solution, previously diluted with 95% ethanol to the SV Minicolumn. Centrifuge the SV Minicolumn assembly for 1 minute at maximum speed**
11. **Empty the Collection Tube as before, and place the SV Minicolumn back in the Collection Tube. Repeat the wash with 500 µL of Membrane Wash Solution, and centrifuge the SV Minicolumn assembly for 5 minutes at maximum speed**
12. **Remove the SV Minicolumn assembly from the centrifuge (not wetting the bottom of the column with the supernatant). Empty the Collection Tube and centrifuge the assembly for 1 minute with the microcentrifuge lid open (or off) to allow ethanol evaporation**
13. **Carefully transfer the SV Minicolumn to a clean 1.5 mL tube. Apply 50 µL of Nuclease-Free Water (at 50ºC) directly to the center of the column, without touching the membrane with the pipette. Incubate at room temperature for 5 minutes**
14. **Centrifuge for 1 minute at 14000 rpm. Discard the SV Minicolumn, and store the tube containing the eluted DNA at 4ºC or -20ºC**

Restriction product purification (from Promega Wizard ® Kit)

1. **Add an equal volume of Membrane Binding Solution to the restriction product tube**
2. **Place one SV Minicolumn in a Collection Tube for each restriction product solution**
3. **Transfer the mixture to the SV Minicolumn assembly and incubate for 1 minute at room temperature**
4. **Centrifuge the SV Minicolumn assembly in a microcentrifuge at max speed for 1 minute. Remove the SV Minicolumn from the Spin Column assembly and discard the liquid in the Collection Tube. Return the SV Minicolumn to the Collection Tube afterwards**
5. **Wash the column by adding 700 µL of Membrane Wash Solution, previously diluted with 95% ethanol to the SV Minicolumn. Centrifuge the SV Minicolumn assembly for 1 minute at maximum speed**
6. **Empty the Collection Tube as before, and place the SV Minicolumn back in the Collection Tube. Repeat the wash with 500 µL of Membrane Wash Solution, and centrifuge the SV Minicolumn assembly for 5 minutes at maximum speed**
7. **Remove the SV Minicolumn assembly from the centrifuge (not wetting the bottom of the column with the supernatant). Empty the Collection Tube and centrifuge the assembly for 1 minute with the microcentrifuge lid open (or off) to allow ethanol evaporation**
8. **Carefully transfer the SV Minicolumn to a clean 1.5 mL tube. Apply 50 µL of Nuclease-Free Water (at 50ºC) directly to the center of the column, without touching the membrane with the pipette. Incubate at room temperature for 5 minutes**
9. **Centrifuge for 1 minute at 14000 rpm. Discard the SV Minicolumn, and store the tube containing the eluted DNA at 4ºC or -20ºC**

IDT GBlocks ® working solutions

1. **Linearize 1 µg vector by restriction digest**
2. **Remove the 5' phosphates from the vector with alkaline phosphatase**
3. **Purify the linearized vector using an agarose gel**
4. **Resuspend the GBlocks gene fragments to a final concentration of 10 ng/µL of water or TE buffer. The amount of DNA in each tube can be checked in the delivery document**
5. **Prepare ends of GBlocks fragments by restriction of 10 µL**
6. **Heat inactivate the enzyme, or column purify the restriction product (depending on the restriction protocol) keeping the digested insert as concentrated as possible**
7. **Ligate 50 ng of vector with a 3 times molar excess of GBlocks in fresh T4 DNA ligase buffer and 400 u of T4 DNA ligase, setting the final volume to 20 µL. Incubate the solution for 2 hours at 16º**
8. **Transform the ligation protocol into competent cells using the Transformation protocol**

PCR (Phusion ®)

Materials Mix

Material	Final concentration
5x Phusion buffer	1x
100% DMSO (with high GC%)	3%
dNTP	0.2 µM
Forward primer	0.5 µM
Reverse primer	0.5 µM
Template DNA	10 ng
Phusion-Polymerase	1 U
dH_2O	Up to 50 µL

PCR machine program

Segment	Cycles	Temperature (ºC)	Time (min)
1	1	98	00:30
2	25	98	00:10
		Tm*	00:30
		72	00:15 per kb of template length
3	1	72	10:00
4	1	4	∞

*Where Tm is the lowest Tm of the primers used

Colony PCR (GoTaq ®)

1. **Make the MasterMix using the materials specified below**
2. **Pick a colony and dip in this mixture and then in 50 µL LB, so the colonies that contain the correct insert can be grown**
3. **Run the PCR using the program specified below**

PCR mix preparation

Material	Final concentration
5x green GoTaq buffer	1x
100% DMSO (with high GC%)	3%
dNTP	0.2 mM
Forward primer	0.5 mM
Reverse	0.5 mM
Template from bacterial colonies	-
GoTaq-Polymerase	1.25 U
dH_2O	Up to 50 µL

PCR machine program

Segment	Cycles	Temperature (ºC)	Time (min)
1	1	98	02:00
2	25	98	00:15
		55	00:30
		72	01:00 per kb of template length
3	1	72	05:00
4	1	4	∞

TOPO TA ® Cloning procedure

Materials

1. **Salt solution,** containing 1.2 M NaCl and 0.06 M MgCl2
2. **Taq polymerase**
3. **dATP solution**
4. **10x buffer**, containing 100 mM Tris-HCl, pH 8.3 (at 42°C), 500 mM KCl, 25 mM MgCl2 and 0.01% gelatin
5. **DNA for transform into the cells**
6. **Competent CaCl2 cells**
7. **SOC medium,** containing 2% Tryptone, 0.5% Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, 20 mM glucose
8. **LB-Agar petri dishes**

Methodology

1. **Get adenine nucleotide at 3', by adding**
    a. **6 uL of DNA**
    b. **1 uL of Taq Polymerase**
    c. **1 uL of dATP**
    d. **1 uL of 10x buffer**
2. **Use 3 uL of this solution and put them in a different tube.**
3. **Add 1 uL of Salt Solution, 2 uL of miliQ water**
4. **Incubate for 10 minutes at room temperature**
5. **2 uL are added into thawed competent cells, and the mixture is incubated for 15 minutes at room temperature**
6. **Heat shock the cells at 42ºC for 30 seconds**
7. **Place the tubes on ice, and add 250 uL of SOC medium to the mixture**
8. **Grow the cells at 37ºC for 1 hour**
9. **Plate the cultures on agar plate, using the necessary antibiotic**

http://www.google.nl/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&ved=0CDEQFjABahUKEwixiM67zcHHAhVEOxQKHQnADyY&url=http%3A%2F%2Ftools.thermofisher.com%2Fcontent%2Fsfs%2Fmanuals%2Ftopota_man.pdf&ei=mv7aVbHTKMT2UImAv7AC&usg=AFQjCNFl0jognsFPzru6PETоemB5eauPWg&sig2=R4P1618Ds-W-meECeGP-sg&cad=rja

Microtiter Plate Assay for Ring-Shaped (*curli* dependent) biofilm[2]

Materials

1. **LB medium**
2. **Lb+rhamnose medium, at different concentrations for induction**
3. **Autoclaved, flat bottomed, transparent 96-well microtiter plates with lids**
4. **Crystal violet solution at 0.1% (w/v) in water**
5. **95% (v/v) ethanol**
6. **Platform shaker**
7. **Plate reader**

Methodology

1. *Make an overnight culture of the bacteria of interest in LB+antibiotic at 37°C*
2. *Dilute each overnight culture by 1:100 into 200 µL of LB-rhamnose in wells of a cell culture treated, flat bottom, transparent 96-well microtiter plates with lids. Cover the plate*
3. *Incubate the plate for ~3 days, leaving enough time for the induction to occur*
4. *Remove the planktonic bacteria from each well by pipetting or shaking out the liquid*
5. *Wash the wells with water to remove the remaining planktonic cells. Submerge the plate in a tray of water and shake out the liquid. One can also add in water and remove the liquid with a multichannel pipet. Repeat this step twice*
6. *Add 210 µL of crystal violet solution into each well and stain for 10 min at RT*
7. *Discard the crystal violet solution, washing the wells three times in a tray or by pipetting (for removing non-specific interactions of the dye)*
8. *Invert the microtiter plate and vigorously tap the plate on a paper tower to remove the remaining water in the well. Air-dry the plate*
9. *Add 200 µL of 95% ethanol into each stained well. Cover the plate and incubate it on a platform shaker at RT for 15 min*
10. *Transfer the 100 µL of liquid onto a non-sterile, flat bottom, transparent 96-well microtiter plate. Measure the OD at 600 nm on a plate reader*

1. ***Zhou, Y., Smith, D. R., Hufnagel, D. A., & Chapman, M. R. (2013). Experimental manipulation of the microbial functional amyloid called curli. In Bacterial cell surfaces (pp. 53-75). Humana Press.***

Isolation using Ni-column purification assay (QIAGEN kit) for extracellular protein tagged with 6xHis [3]

Materials

1. **Wash buffer NPI-20**
2. **Elution Buffer NPI-500**
3. **Lysis buffer NPI-10**

Methodology

1. *Inoculate 10 mL of LB medium containing the appropriate antibiotics with a fresh bacterial colony harboring the expression plasmid. Grow at 37ºC overnight*
2. *Dilute the non-induced overnight culture 1:60 with fresh LB medium containing the appropriate antibiotics. Grow at 37ºC while shaking until the OD_{600} reaches 0.6*
3. *Add Rhamnose (induction) to the desired final concentration for induction, and grow at 37ºC for the appropriate induction time*
4. *Harvest the cells by centrifugation at 4000 g for 15 min*
5. *Transfer the supernatant to another tube and discard the pellet. Keep a part of the supernatant for SDS-PAGE analysis, if needed*
6. *Equilibrate the Ni-NTA spin column with 600 µL Buffer NPI-10. Centrifuge for 2 min at 2900 rpm (890 g)*
7. *Make the supernatant solution, by setting the final solution to contain 200mM NaCl, 1mM PMSF, and 20mM imidazole. The pH is adjusted to 7.8, using 1M K2HPO4 solution*
8. *Load up to 600 µL of the supernatant solution containing the 6xHis-tagged protein onto the pre-equilibrated Ni-NTA spin column. Centrifuge for 5 min at 1600 rpm (270 g) and collect the flow-through*
9. *Wash the Ni-NTA spin column twice with 600 µL Buffer NPI-20. Centrifuge for 2 min at 2900 rpm (890 g)*
10. *Elute the protein twice with 300 µL of Buffer NPI-500. Centrifuge for 2 min at 2900 rpm (890 g) and collect the eluate*

Isolation using Ni-column purification assay (QIAGEN kit) for intracellular protein tagged with 6xHis [3]

Materials

1. **Wash buffer NPI-20**
2. **Elution Buffer NPI-500**
3. **Lysis buffer NPI-10**
4. **Lysozyme stock solution 10 mg/mL in water**
5. **Benzonase ® Endonuclease 25 U/µL**

Methodology

1. *Inoculate 10 mL of LB medium containing the appropriate antibiotics with a fresh bacterial colony harboring the expression plasmid. Grow at 37ºC overnight*
2. *Dilute the non-induced overnight culture 1:60 with fresh LB medium containing the appropriate antibiotics. Grow at 37ºC while shaking until the OD_{600} reaches 0.6*
3. *Add Rhamnose (induction) to the desired final concentration for induction, and grow at 37ºC for the appropriate induction time*
4. *Harvest the cells by centrifugation at 4000 g for 15 min*
5. *Discard the supernatant and keep the pellet. Then resuspend the pellet in 630 µL of Lysis buffer (NPI10). Add 70 µL of Lysozyme stock solution (10 mg/mL) and add 3 units/mL of Benzonase®*
6. *Incubate on ice for 15-30 min*
7. *Centrifuge the lysate at 12000 g for 15-30 min at 4ºC. Collect the supernatant*
8. *Equilibrate the Ni-NTA spin column with 600 µL Buffer NPI-10. Centrifuge for 2 min at 2900 rpm (890 g)*
9. *Load up to 600 µL of the cleared lysate solution containing the 6xHis-tagged protein onto the pre-equilibrated Ni-NTA spin column. Centrifuge for 5 min at 1600 rpm (270 g) and collect the flow-through*
10. *Wash the Ni-NTA spin column twice with 600 µL Buffer NPI-20. Centrifuge for 2 min at 2900 rpm (890 g)*
11. *Elute the protein twice with 300 µL of Buffer NPI-500. Centrifuge for 2 min at 2900 rpm (890 g) and collect the eluate*

Congo Red (CR) Assay to quantify curli generation[0]

Materials

1. **LB agar plates**
2. **LB liquid medium**
3. **Congo Red (CR) stock: Dissolve 1 g of Congo Red in 100 mL of water and sterilize by filtering. Store at 4ºC**
4. **Chloramphenicol stock solution (35 mg/mL)**
5. **Rhamnose (0.1-1% w/v)**
6. **IPTG (4mM)**

Methodology

1. Streak out the cells from a -80ºC cryostock onto LB+agar plate. Pick grown colonies and grow them overnight in liquid LB + antibiotic
2. Transfer 1 mL of the overnight culture in 30 mL LB+antibiotic, and keep it growing while shaking
3. Once the OD600 of the culture is around 0.4, induce the cultures with the desired amount of inductor (Rhamnose or IPTG). Wait 2 days after the induction
4. Take 1 mL of every sample and measure the OD600, using LB+CAM as blank
5. Add Congo Red to a final concentration of 20 µg/mL, mix and incubate for 5 min at room temperature
6. Centrifuge at 14000 rpm for 5 minutes
7. Take the supernatant and measure it at 480 nm (or the peak in the spectrophotometric absorption curve), using LB+CAM+Congo Red as a reference

0. Zhou, Y., Smith, D. R., Hufnagel, D. A., & Chapman, M. R. (2013). Experimental manipulation of the microbial functional amyloid called curli. In Bacterial cell surfaces (pp. 53-75). Humana Press.

Plate reader Assay to quantify GFP intensity for testing plasmid performance and kinetic parameters

1. Grow a ΔcsgA - csgA and ΔcsgA – csgA-I13521 strain at 37°C overnight in LB medium.
2. Measure the OD600. As the OD600 will probably be high (>2.5), make a ~50x dilution and grow the liquid cultures at 37°C for ~2h.
3. Measure the OD600 again. If the OD600 is between 0.4 and 0.6 (exponential phase), continue to step 4.
4. Write down the OD600 that was obtained. Make a dilution to an OD600 of 0.05.
5. Fill the wells with different amounts of rhamnose (from 50 g / L stock) and sterile water.
6. Fill the wells with 180 ul LB + cells, or solely 180 ul LB.
7. When all the wells are filled, measure the fluorescence and OD600 in a plate reader in time (at a temperature of 30°C).

Attachment assay for testing biofilm adhesion strength on hydroxyapatite using *Bos Taurus* dental parts

Characterization of the surface of the samples

1. A tooth sample is cleaned and disinfected by ethanol bath overnight
2. The tooth is then carefully broken down into smaller parts
3. Three of these pieces are then selected based on the size and shape, taking the three cubical pieces with more resemblance
4. Keep the samples in ethanol, so they stay sterile before their use

Biofilm formation on the surface

1. Cells csgA+Hydroxyapatite+RFP affinity tag positive after induction are taken from a cryostock/plate and cultured overnight in LB media. It is important to also grow two cultures that can be used as a control, making the total number of samples 3
2. The cultures are then dropped in a sterile, empty 12-wells plate. After that, the dental samples are placed in the middle of one well
3. Next to the dental sample, Rhamnose solution is added to the dish to a final concentration of 0.5%. After this step, the plate is left for induction for 20-40 hours

Hydroxyapatite-tag strength test

1. The fluorescence of the samples is then measured Typhoon fluorescence reader, after two immersions in water
2. The samples are then placed in a tube and rinsed with 1 ml of ethanol. The mixture is left 5 minutes so all the cells can detach from the tooth
3. Then, the ethanol solutions are taken from the tube and placed in a 96-well plate, and the RFP intensity is measured using a fluorescence plate reader

Western Blotting

1. Grow a ΔcsgA - csgA and ΔcsgA – csgA-His strain at 37°C overnight in LB medium.
2. Measure the OD600. Make a ~50x dilution and grow the liquid cultures at 37°C for 1.5h.
3. Measure the OD600 again. If the OD600 is between 0.35 and 0.6 (exponential phase) , the induction can be made.
4. Induce the cells with Rhamnose (from 50 g / L stock) and sterile water. You need to have a final concentration of 0.5 wt% Rhamnose. The induction has to be performed at 30°C.
5. After 5 hours of induction, measure the OD600 and disrupt the cells.
6. Run SDS-PAGE gel with protein marker and samples (use pre-stained protein marker)
7. Transfer protein from gel to membrane:
   - Soak 2 x filter thick paper and the gel in transfer buffer.
   - Soak 1 x PVDF membrane in 100% methanol, then water, then transfer buffer or use cellulose membranes soaked only in transfer buffer.
   - Make sandwich: filter bottom paper, membrane, gel (upside down), filter top paper.
   - Roll out bubbles with glass pipette.
   - Attach top electrode plate.
   - Transfer 40 min at 15V.
8. Development blot with Supersignal West pico kit (Pierce):
   - Block blot membrane in 5% milk or BSA in TBS-T, on orbital shaker 1 hour (RT) to overnight (cold room).
   - Add anti-His-HRP antibody (dilution 1:5000) in blocking solution and incubate on orbital shaker for 1h at room temperature.
   - Wash 4 x 10min with TBS-T on orbital shaker, RT.
   - Detection (Supersignal West pico kit)
     - ✓ Put blot membrane on Saran wrap
     - ✓ Mix 1.5 ml detection reagent 1 and 1.5 ml detection reagent just before use
     - ✓ Pipette 3 mL of mix detection reagent on blot (make sure there is even distribution on the membrane)
     - ✓ Incubate 5 minutes at RT in dark
     - ✓ detect chemiluminescence with CCD camera (Biorad Imager in Biobrick)

Spinning Disk Sample preparation for visualization of bacterial biofilm layers

Materials

- **1 % w/v Sodium alginate**
- **0.1M CaCl2**
- **0.1M sodium citrate (monobasic)**
- **5 % w/v L-Rhamnose**

Preparation bio-ink

1. Grow cells overnight in ~ 5mL LB + CAM.
2. Spin down cells at 4000 rpm for 3 min
3. Discard supernatant
4. Resuspend pellet in 100 µL LB and transfer to 1.5 mL tube
5. Add 55.6 µL 5% w/v rhamnose to get a 0.5% rhamnose concentration
6. Add 400 µL alginate and vortex

Printing

7. Make line with pipet (~10-20 µL each) on plasma cleaned cover slip
8. Place cover slip onto a tissue
9. Add CaCl2 by dripping it on the alginate lines with pipet
10. Place the cover slip in vertical position on the tissue to remove most of the CaCl2
11. Retake above steps for the second line

Store

12. Put a tissue in a petri dish and make it wet
13. Put a piece of parafilm on top and place the sample on top of the parafilm
14. Store at room temperature
15. After induction with rhamnose wait at least 24h before dissolving the hydro gel

Dissolving gel

16. Place cover slip onto a tissue
17. Add sodium citrate by dripping it on the alginate lines with pipet
18. Place the cover slip in vertical position on the tissue to remove most of the sodium citrate
19. Store sample again in the petri dish

Transmission Electron Microscopy (TEM) Sample preparation for visualization of bacterial amyloid curli

1. Make an overnight culture of the cells containing the plasmid with csgA, using LB+CAM, at 37ºC and 220 RPM
2. Dilute the cultures to a final OD600 of 0.05, by taking a small part of the overnight culture and adding LB+CAM
3. Let the cultures grow at 37ºC and 220 RPM until the OD600 reaches a value between 0.4-0.6
4. Induce the cultures with different concentrations of L-rhamnose, from 0% to 1%
5. Add a small volume (1%) of the sample directly on the grid of the TEM
6. Take pictures of the visualized bacteria

Blue Fluorescent Protein (BFP) assay

1. Grow BFP_SpyCatcher_His (TOP10) and empty TOP10 strain at 37°C overnight in LB medium.
2. Measure the OD600. As the OD600 will probably be high (>2.5), make a ~50x dilution and grow the liquid cultures at 37°C for ~2h.
3. Measure the OD600 again. If the OD600 is between 0.4 and 0.6 (exponential phase), continue to step 4.
4. Fill the wells with different amounts of arabinose (from 100 g / L stock) and sterile water.
5. Fill the wells with 180 ul LB + cells, or solely 180 ul LB.
6. When all the wells are filled, measure the fluorescence and OD600 in a plate reader in time (at a temperature of 30°C).

ID	Arabinose concentration	Arabinose concentration	Construct type	Number of wells	Arabinose (100g/L)	Water	LB + cells
1	0 wt%	0 g/L	BFP_Spy	3x (+1 without cells)	0 ul	20 ul	180 ul
2	0.2 wt%	2 g/L	BFP_Spy	3x (+1 without cells)	4 ul	16 ul	180 ul
3	0.5 wt%	5 g/L	BFP_Spy	3x (+1 without cells)	10 ul	10 ul	180 ul
4	1.0 wt%	10 g/L	BFP_Spy	3x (+1 without cells)	20 ul	0 ul	180 ul
5	0 wt%	0 g/L	TOP10	3x (+1 without cells)	0 ul	20 ul	180 ul
6	0.2 wt%	2 g/L	TOP10	3x (+1 without cells)	4 ul	16 ul	180 ul
7	0.5 wt%	5 g/L	TOP10	3x (+1 without cells)	10 ul	10 ul	180 ul
8	1.0 wt%	10 g/L	TOP10	3x (+1 without cells)	20 ul	0 ul	180 ul

Volume of 1 well = 200uL. Number of wells filled: 44.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0.2%	0.2%	0.5%	0.5%	1.0%	1.0%	0%	0%	LB	LB	LB	LB
B												
C	1	1	1	1	2	2	2	2	3	3	3	3
D												
E	4	4	4	4	6	6	6	6	5	5	5	5
F												
G	7	7	7	7	8	8	8	8-				
H												

The minus denotes the control samples without the cells